

G¹ 35. (twice amended) A modified pneumolysin polypeptide, wherein the modification of the polypeptide comprises substituting at least one amino acid of SEQ ID NO:3, wherein said substitution is at a position selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, ^{and} wherein when said modified pneumolysin polypeptide possesses ^{at least one substitution at} only one substitution, said one substitution ^{is selected from the group consisting of positions 61, 148, and 195, and wherein said modified pneumolysin is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.}

G² 53. (twice amended) A modified pneumolysin polypeptide, wherein the polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, has attenuated hemolytic activity, and wherein the modification of the polypeptide is obtained by randomly mutating a nucleic acid molecule encoding a pneumolysin polypeptide.

G³ 62. (twice amended) A vaccine comprising the polypeptide according to claim 35 and a pharmaceutically acceptable carrier.

G⁴ 64. (twice amended) The vaccine according to claim 63, wherein the bacterial polysaccharide is from a bacterium selected from the group consisting of *Haemophilus influenzae* type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus type Ia, Ib, II, III, V, or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.

G⁵ 65. (amended) The polypeptide according to claim 35, wherein said modified pneumolysin polypeptide having only one amino acid substitution is a proline or hydroxyproline substitution at position 61.

66. (amended) The polypeptide according to claim 35, wherein said modified pneumolysin polypeptide having only one amino acid substitution is a lysine, arginine or histidine substitution at position 148.

67. (amended) The polypeptide according to claim 35, wherein said modified pneumolysin polypeptide having only one amino acid substitution is a leucine, glycine, alanine, valine or isoleucine substitution at position 195.

68. (amended) The polypeptide according to claim 35, wherein said amino acid substitution is an arginine, valine, glutamic acid, or serine substitution at position 243. ✓

69. (amended) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 17, 18, 61, 66 and 101. ✓

70. (amended) The polypeptide according to claim 69, wherein the substitutions consist of arginine at position 17, asparagine at position 18, proline at position 61, tyrosine at position 66, and threonine at position 101.

71. (amended) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 41, 172, 195 and 255. ✓

72. (amended) The polypeptide according to claim 71, wherein the substitutions consist of glycine at position 41, alanine at position 172, isoleucine at position 195, and glycine at position 255.

73. (amended) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 63, 127, 128 and 148. ✓

74. (amended) The polypeptide according to claim 73, wherein the substitutions consist of serine at position 63, glutamic acid at position 127, histidine at position 128, and lysine at position 148.

75. (amended) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 33, 46, 83, 239 and 257. ✓

76. (amended) The polypeptide according to claim 75, wherein the substitutions consist of threonine at position 33, threonine at position 46, serine at position 83, arginine at position 239 and glycine at position 257. ✓

77. (amended) The polypeptide according to claim 75, wherein the substitutions at positions 33, 46 and 83 are selected from the group consisting of a serine, threonine, asparagine, glutamine, tyrosine and cysteine; the substitution at position 239 is selected from the group consisting of a lysine, arginine and histidine; and the substitution at position 257 is selected from the group consisting of a leucine, glycine, alanine, isoleucine and valine. ✓

78. (amended) The polypeptide according to claim 35, wherein the modification of the polypeptide comprises a combination of substitutions at positions 45, 102, 189 and 195.

79. (amended) The polypeptide according to claim 78, wherein the combination of substitutions consists of alanine at position 45, glycine at position 102, arginine at position 189, and valine at position 195.

80. (additional) A modified pneumolysin polypeptide, wherein the modification of the polypeptide comprises substituting at least one amino acid sequence having SEQ ID NO:3, wherein said substitution is at a position selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, wherein when said modified pneumolysin polypeptide possesses only one substitution, said one substitution is selected from the group consisting of positions 61, 148, and 195, wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity, wherein the amino acid substitution at position 17 is arginine; wherein the amino acid substitution at position 18 is asparagine; wherein the amino acid substitution at positions 33, 46, and 83 is selected from the group consisting of serine, threonine, asparagine, glutamine, tyrosine, and cysteine; wherein the amino acid substitution at position 41 is glycine; wherein the amino acid substitution at position 45 is alanine; wherein the amino acid substitution at position 61 is a proline or hydroxyproline; wherein the amino acid substitution at position 63 is serine; wherein the amino acid substitution at position 66 is tyrosine; wherein the amino acid substitution at position 101 is threonine; wherein the amino acid substitution at position 102 is glycine; wherein the amino acid substitution at position 127 is glutamic acid; wherein the amino acid substitution at position 148 is selected from the group consisting of lysine, arginine and histidine; wherein the amino acid substitution at position 172 is alanine; wherein the amino acid substitution at position 189 is arginine; wherein the amino acid substitution at position 195 is selected from the group consisting of leucine, glycine, alanine, valine and isoleucine; wherein the amino acid substitution at position 239 is selected from the group consisting of lysine, arginine, and histidine; wherein the amino acid substitution at position 243 is selected from the group consisting of arginine, valine, glutamic acid, and serine; wherein the amino acid substitution at position 255 is glycine; and wherein the amino acid substitution at position 257 is selected from the group consisting of leucine, glycine, alanine, isoleucine, and valine.